

PCT/EP 99/08004
09/830354

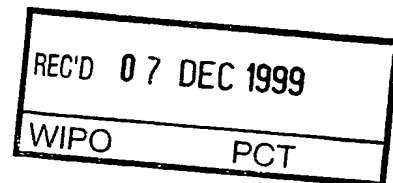


Eur päisches
Patentamt

European
Patent Office

Office européen
des brevets

EP 99/08004



Bescheinigung

Certificate

Attestation

4

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

98250380.7

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
THE HAGUE,
LA HAYE, LE

30/11/99



Eur päisches.
Patentamt

European
Patent Office

Office eur péen
des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: 98250380.7

Anmeldetag:
Date of filing: 28/10/98
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
Ikonomidou, Hrissanthi
13505 Berlin
GERMANY

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:

New use of glutamate antagonists for the treatment of cancer

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:
State:
Pays:

Tag:
Date:
Date:

Aktenzeichen:
File no.
Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:

A61K31/435, A61K31/55, A61K31/495

Am Anmeldetag benannte Vertragsstaaten:
Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:

Pages 1 to 19 of the description were missing at the date of filing of the application

Tumor cell line: SK-N-AS, obtained from human neuroblastoma (European Collection of Cell Cultures). Tumor cells were incubated in 96 well plates in culture medium in a CO₂-incubator at 37°C.

Exposure to agents: Tumor cells were exposed to the following agents and concentrations: Cisplatin (10 µM), Vinblastin (1 µM), Thiotepa (10 µM) alone or in combination with MK 801 (10µM), GYKI 52466 (10 µM) or NBQX (10 µM).

Assessment of tumoricidal effect: Quantitation of cell death was performed at 24, 48 or 72 hrs after exposure to the agents of interest following staining of the cells with trypan blue. This stain is taken up by necrotic cells. The numbers of necrotic cells/100 counted cells served as quantitative measure for tumoricidal effect (% of tumor cell death). For each agent or combination of agents and each time point 5 wells were analysed. Comparisons between groups was performed by means of Student's t-test.

Results

Each of the cytostatic agents tested produced a significant tumoricidal effect on its own at the concentrations used, which reached a peak for all three agents after 72 hrs exposure time. The NMDA antagonist MK 801 and the AMPA antagonists GYKI 52466 and NBQX significantly ($P<0.05-0.001$) potentiated tumoricidal effect of cisplatin, vinblastin and thiotepa when used in combination with the cytostatic agents. This potentiation of the tumoricidal effect of the cytostatic agents by the NMDA and the AMPA antagonists was most prominent after an exposure time of 72 hrs for cisplatin and thiotepa and 48 hrs for vinblastin (Figs. 1-3).

CLAIMS

1. The use of an inhibitor of the interaction of glutamate with the AMPA receptor complex in the manufacture of a medicament for treating cancer.
2. The use according to claim 1, wherein the type of cancer includes all kinds of cancer.

3. The use according to any preceding claim wherein the inhibitor is an antagonist of the binding of glutamate to the AMPA receptor.
4. The use according to any preceding claim, wherein the inhibitor is an L-glutamate derivative, an α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate derivative, acid amide derivatives, amino alkanolic acid, amino-phenyl-acetic acid, amino- or desamino- 2,3-benzodiazepine, 2,3-benzodiazepin-4-one, alkoxy-phenyl-benzodiazepine, acetyl-aminophenyl-dihydro-methyl-dioxolo-benzodiazepine, benzothiadiazine, decahydroisoquinoline, β -carboline-3-carboxylic acid, fused cycloalkylquinoxalinedione, 4-hydroxypyrrolone, 4-hydroxy-pyrrolo-pyridazinone, indeno-pyrazine-carboxylic acid, indeno-pyrazinone, indoloneoxime, indolo-pyrazinone, indolo-pyrazinone, imidazo-pyrazinone, imidazolo-quinoxalinone, isatine, isatinoxime, oxadiazole, phenyl-azolophthalazine, phenylpyridazino-indole-1,4-dione, quinoline, quinolone, quinolonone, nitroquinolone, quinoxaline, quinoxalinedione, quinazolinone, 4-hydroxy-pyrrolo-pyridazinone, phenyl-azolophthalazine or sulphamate derivatives.
5. The use according to any of claims 1 to 4, wherein the inhibitor is L-glutamic acid diethylester, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), 6,7-dinitro-quinoxaline-2,3-dione (DNQX), 6-nitro-7-cyano-quinoxaline-2,3-dione (CNQX), 6-(1-imidazolyl)-7-nitro-quinoxaline-2,3(1H, 4H)-dione (YM90K), [2,3-dioxo-7(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxaliny]-acetic acid monohydrate (YM872), (3RS,4aRS,6RS,8aRS)-6-(2-(1H-tetrazole-5-yl)ethyl)-decahydroiso-quinoline-3-carboxylic acid (LY293558), 9-methyl-amino-6-nitro-hexahydro-benzo(F) quinoxalinedione (PNQX), 8-methyl-5-(4-(N,N-dimethylsulphamoyl)phenyl)-6,7,8,9-tetra-hydro-1H-pyrrolo[3,2h]-isoquinoline-2,3-dione-3-O-(3-hydroxybutyric acid-2-yl)oxime (NS 1209), 6,7-dichloro-2-(1H)-quinolinone-3-phosphonate (S 17625-2), [1,2,3,4-tetrahydro-7-morpholinyl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-1-yl]methyl-phosphonate (ZK200775), 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine (GYKI52466), topiramate and 5-{2-[2-(N,N-dimethylamino)ethyl]oxy-phenyl}-3-phenyl-1,2,4-oxadiazol, 1-(4-aminophenyl)-3-methylcarbamoyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 53655).
6. The use according to any of claims 1 to 2, wherein the inhibitor is an AMPA receptor channel blocker.

7. The use according to claim 6, wherein the AMPA receptor channel blocker is fluorowillardiine, Joro spider toxin, NSTX spider toxin, argitoxin, or their derivatives.
8. The use of an inhibitor of the interaction of glutamate with the KA receptor complex in the manufacture of a medicament for treating cancer.
9. The use according to claim 8, wherein the type of cancer includes all kinds of cancer.
10. The use according to claim 8 and 9 wherein the inhibitor is an antagonist of the binding of glutamate to the KA receptor.
11. The use according to claims 8 to 10, wherein the inhibitor is an L-glutamate derivative, kainic acid derivative, domoic acid derivative, acid amide derivative, aminoalkanoic acid derivative, aminophenyl(alkyl)acetic acid derivative, isatine, quinoxalinedione, fused cycloalkylquinoxalinedione, imidazolo-quinoxalinone, phenyl-azolophthalazine, pyridothiazines, quinazoline, quinazolinedione, quinolinone, 4-phosphonoalkyl-quinolinone, quinoxalinedione, or sulphamate derivative.
12. The use according to any of claims 8 to 11, wherein the inhibitor is 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), 6,7-dinitro-quinoxaline-2,3-dione (DNQX), 6-nitro-7-cyano-quinoxaline-2,3-dione (CNQX), 6-(1-imidazolyl)-7-nitro-quinoxaline-2,3(1H,4H)-dione (YM90K), [2,3-dioxo-7(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxaliny]-acetic acid monohydrate (YM872), (3RS,4aRS,6RS,8aRS)-6-(2-(1H-tetrazole-5-yl)ethyl)-decahydroisoquinoline-3-carboxylic acid (LY293558), 9-methyl-amino-6-nitro-hexahydrobenzo(F)quinoxalinedione (PNQX), 8-methyl-5-(4-(N,N-dimethylsulphamoyl)phenyl)-6,7,8,9-tetrahydro-1H-pyrrolo[3,2h]-isoquinoline-2,3-dione-3-O-(3-hydroxybutyric acid-2-yl)oxime (NS 1209), 6,7-dichloro-2-(1H)-quinolinone-3-phosphonate (S17625-2), [1,2,3,4-tetrahydro-7-morpholinyl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-1-yl]methyl-phosphonate (ZK200775), 1-(aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466), γ -D-glutamylaminomethylsulphonate (GAMS), γ -D-glutamylglycine.
13. The use according to any of claims 8 to 9, wherein the inhibitor is an KA receptor channel blocker.

14. The use according to claim 13, wherein the KA receptor channel blocker is fluorowillardiine, Joro spider toxin, NSTX spider toxin, argiotoxin, or their derivatives.
15. The use of an inhibitor of the interaction of glutamate with the NMDA/glycine/polyamine receptor/ion channel complex in the manufacture of a medicament for treating cancer.
16. The use according to claim 1, wherein the type of cancer includes all kinds of cancer.
17. The use according to claims 15 to 16 wherein the inhibitor is an antagonist of the binding of glutamate to the NMDA receptor or NMDA receptor associated binding sites such as e.g. glycine or polyamine binding sites.
18. The use according to claims 15 to 17, wherein the inhibitor is an L-glutamate derivative, a 4-hydroxy-3-nitro-1,2-dihydroquinolon-2-one derivative, an indole derivative, a benzo-thiadiazine dioxide derivative, an indeno(1,2-b)pyrazin-3-one or corresponding 2,3-dione, a quinoline derivative, an ethyl(phenyl-carbamoyl)-ethenyl)dichloroindole carboxylate, a thienopyrazine 2,3-dione derivative, a 2-(2,3-dicarboxycyclopropyl) glycine, a 2-amino-3-substituted phenyl propionic acid derivative, 1-carboxyalkylquinoxaline-2,3(1H,4H)dione derivative, a thienyl-glycine derivative, a benzo-fused azacyclic compounds, an indole derivatives, a tricyclic quinoxaline-diene derivative, a 3-hydroxy anthranilic acid and salts, a decahydroisoquinoline compound, a tri- or tert-substituted guanidine derivatives, a D- or L-tryptophan derivative, a tetrazolyl(alkyl)-cyclohexyl-aminoacid derivative, an octahydrophenanthrene derivative, a benzomorphan compound, a piperazinyl or piperidinyl-alkyl substituted isoxazole derivative, a decahydroisoquinoline-3-carboxylic ester or amide preparation, a compounds based on Conantokin-G peptide, a 3-heterocyclyl-alkyl-benzopyran-2-one derivative, a phosphono-alkyl imidazo-pyrimidine carboxylic acid derivative, amantidine, memantine, rimantidine, a histogranin peptide or analogue, a nitrobenzoic acid derivative, e.g. 4-((2-methoxycarbonyl-4-nitrophenyl)methyl)piperazine carboxylic acid, a diamine derivative with selective sigma receptor affinity, remacemide (2-amino-N-(1,2-diphenyl-1-methylethyl)acetamide), a phosphono-alkylidene- or phosphono-alkoxy-imino-piperidine acid, a benzothiadiazine carboxylic acid derivative, a dihydro-benzothiadiazine dioxide carboxylic acid derivative, a 4-hydroxy 2(H) pyrrolone derivative, a quinoxaline derivative, a tetrahydro-imidazo (1,2-a) pyrimidines or its salt, a alpha-amino acid, a 4-hydroxy-pyrrolo(1,2-

b)pyridazin-2(1H)-one derivative, a nitroquinolone derivative, a 3-aryl-substd 2(1H)quinolone, a 2(1H)-quinolone, a phosphonic acid quinoline-2-carboxylic acid derivative, its per hydro quinoline derivative or salt, a benzimidazole(s) carrying 2 acidic groups, an N,N'-disubstituted guanidine derivative, a tricyclic quinoxaline dione, a 2-(2,3-dicarboxycyclopropyl) glycine stereoisomer, pregnenolone sulphate or one of its derivative, an isatine derivative, a 3-amino-indolyl-derivative, 2-phenyl-1,3-propanediol dicarbamate (felbamate), a benzomorphan derivative, a dihydrothienopyridine derivative, an enantiomer of (aminophenyl)-heteroaryl ethylamine, a pyridazine-dione derivative, a 2H-1-benzopyran-2-one compound, a 4-sulphonylamino-quinoline derivative, a R(plus)-3-amino-1-hydroxy-pyrrolidine-2-one, a 2-carboxy indole, a substd. imino-methano dibenzo (A,D)cycloheptene derivative, an indole-hydrazone, a piperazine derivative, a 4,6-disubstituted tryptophan and kynurenine derivative, a fluorenamine compound, a diketo-pyrido pyrazine derivative or its salts, a 2-amino-3,4-dioxo-1-cyclobutene derivative, a 2-acyl-amido derivative of 3,4-dihydro-3-oxo-quinoxaline, a benzimidazole phosphono-aminoacid derivative, a quinoxaline phosphono-aminoacid derivative, a piperazine, piperidine or pyrrolidone derivative, ist salts and isomeric forms including stereoisomers, a 4-hydroxy-2(1H)-quinolinone derivative, ist salts and prodrugs, a fused pyrazine derivative, a 2-phenyl or 2-thienyl-(2)-piperidine derivative, a 3-amido or 3-sulphamido-indolyl derivative, a 3-aryl-4-hydroxy-2-(1H)-quinolone derivative, a 2-heterocyclyl-2-hydroxy-ethylamine derivative, a 1-aryl-2-aminomethyl pyrrolidine, its optical isomers and acid-addn. salts, a 4,6-dihalo indole-2-carboxylic acid derivative, a cyclic amino-hydroxamate derivative, a tetracyclic amine derivative, a 2,4-dioxo-1,2,3,4-tetrahydroquinoline derivative, a 2,4-dioxo-1,2,3,4-tetrahydroquinoline derivative, a 3-phosphonopiperidine and p-pyrrolidine derivative, a benzothieno (2,3-B)-pyrazine-2,3-(1H,4H)-dione, a spiro dibenzosuberane derivative, a benzomorphan derivative, a preparation of 3,4-disubstituted 2-isoxazoline(s) and isoxazoles(s), a 3-indolyl thio-acetate derivative, an arginine-derived nitric oxide biosynthesis inhibitor, a dicyclic amine derivative, a spiroisindole derivative, an imidazo(1,2-A)-pyridinylalkyl compound, a 1,2,3,4-tetrahydro-9H-pyrido indole or benzothiophene derivative, an indole-2,3-dione-3-oxime derivative, a 1-aryl-2-(aminomethyl)cyclopropanecarboxamide derivative, a 4-phosphono-2-amino-alkenoic acid derivative, a naphthopyran derivative, a beta-ketone, a beta oxime or beta hydrazine phosphonate, a topa quinone aminoacid, kynurenic acid or a derivative, a quinoline- or thienopyridine-carboxylic acid derivative, a 10,5-(imino-methano)-10,11-dihydro-5H-dibenzo(A,D)cycloheptene or a derivative, a bicyclic amino-hydroxamate derivative, an indole-2-carboxylic acid derivative, a substituted adamantane derivative, a benzobicycloalkane derivative, a 2,4-disubstituted-1,2,3,4-tetrahydro-quinoline derivative, a dihydro-alkyl-substituted-

(immunomethano)-5H-dibenzo-cycloheptene, an aryl cyclohexylamine, an N-substd. benzobicycloalkane amine, an isoquinoline phosphonate derivative, an N,N'-disubstd.-guanidine compound, a phosphonopropenyl piperidine carboxylic acid compound, (2R,3S,4S)-alpha-carboxy-cyclo-propyl-glycine, a pyrrolidine derivative, a dihydroxy-fused heterocyclyl quinoxaline derivative, a hydrogenated derivative of MK 801 and analogues, a 5-substd.10,11-dihydro 5H-dibenzo (a,d) cycloheptene 5,10-imine, an 11-Exo-hydroxy MK 801 preparation including electrochemical cyclisation step to form 5,10-imine bridge in 5-methyl 5-oxyamino 5H-dibenzo (A,D) cycloheptene, a tetra hydro-isoquinoline or 2-benzazepine derivative, an N-3-phenyl-propionyl-substd. spermine or related polyamine derivative, a 4a-amino-fluorene compound or a heterocyclic analogue, a cyclooctane-imine derivative, a R-3-amino-1-hydroxy pyrrolidin-2-one or methionine hydroxamate, a 10,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine compound, a polyhydro-10,11-dihydro-5H-benzo(a,d)cyclohepten-5,10 imine derivative, a 4-oxo-1,4-dihydroquinoline compound with 2-acidic groups, a heterocyclyl-alkene-phosphonic acid compound, a phosphono gp-containing pyridine 2-carboxylic acid, an alpha-amino-alpha-(3-alkylphenyl)alkyl ethanoic acid, its esters or amides, a 10,11-dihydro-5H-dibenzo-A,D-cyclohepten-5,10-imine compound, a phosphorus containing unsaturated amino acid or its salts, a 5 Substd.-1-,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine or analogue, a heterocyclic phosphonic acid derivative or its salt, a substituted 4-(amino-carbonyl-amino)quinoline derivative, a tricyclic quinoxaline derivative, a butyryl-tyrosine spermine or one of its analogue, a tri- or tetra-substituted guanidine, a quinoxalinyllalkyl-aminoalkane phosphonic acid derivative, a 2-(aminophenyl)-3-(2-carboxy-indol-3-yl)-propenoic acid derivative, a 6-piperidinylpropionyl-2(3H)-benzoxazolone derivative, 6-(3-[4-(4-fluorobenzyl)piperidin-1-yl]propionyl)-3H-benzoxazol-2-one or one of its salts, an imidazo(1,2-a)pyridine compound, a tetrahydroquinoline derivative or one of its salts, a 2-methyl-5,8-substituted 2,3,4,5-tetra- or 2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b]indole, a 3-aminoindolyl compound, a 6-pyrrolyl-quinoxaline-2,3-dione derivative, an imidazolyl-(mercaptoalkyl)-quinoxaline-dione compound, a 3-amidoindolyl derivative, a heterocyclyl-imidazolo-quinoxalinone compound, a naphthyl-substituted alpha-amino acid derivative, a 5-heteroaryl-2,3-quinoxaline-dione derivative, a quinoxaline derivative, a 5H,10H-imidazo indeno 4-pyrazinone derivative, a hydroxy-(aryl-substituted phenyl)-quinolone compound, an imidazo indolo pyrazinone derivative, a ((phenyl-amino)-(m)ethyl)-pyridine derivative, a tetrahydro-isoquinoline derivative, a 4-substituted piperidine analogue, a 2-substituted piperidine derivative, a tri- or tetra-substituted guanidine derivative, a 3-Hydroxy-4-imidazolidinone, a 3-aminoquinoxalin-2-one derivative, rapamycin or a derivative e.g. 1,3-Diels Alder adduct with phenyl-triazoline-dione, 1-

amino-1-cyclobutanecarboxylic acid, a thiamorphinan derivative, a pyrido[4,3-b]indole derivative, 4-phenyl carbamoyl methylene tetrahydro quinoline-2-carboxylic acid or a derivative thereof, (3R,4S)-3-(4-(4-fluorophenyl)-4-hydroxy-piperidin-1-yl)-chroman-4,7-diol, a phenol derivative, an indeno-pyrazin-4-one, a 2,3-dioxo-1,2,4,5-tetrahydro-quinoxalinyll derivative, a 4,5-bridged quinoxalinedione or quinolone, (1S,2S)-1-(4-hydroxyphenyl)2-(4-hydroxy 4-phenyl piperidin-1-yl) 1-propanol methane sulphonate trihydrate, a 4-sulphanimide-quinoline derivative, a methanobenzocyclodecen-13-amine compound, a derivatives of pregnenolone sulphate, a quinoxalinyll-(alkane,alkene,or alkyne)-phosphonic acid or one of ist esters, a diarylalkylamine related to spider and wasp venom toxins, a piperazine R-alpha-carboxylic acid derivative, an imidazo-indeno-pyrazin-4-one derivative, a pyridazino-quinoline derivative, a 1-substd. or 1,3-di-substd. 1,3-diaryl-guanidine compound, an aza-cycloalkyl-fused quinoxaline-dione, a 3-substd. 2-carboxy-indole derivative or intermediate, a (2R)-N-trityl-4-oxo-5-(dimethyl phosphono)-norvalinate ester, a kynurenic acid derivative, an indole carboxylic acid derivative, a 6-(tetrazolyl or isoxazolyl)-decahydroisoquinoline-3-carboxylic acid derivative, a phenyl- or pyridinyl-thienopyridinone derivative, a fused cycloalkyl-quinoxaline-dione derivative, a pyridazino-quinoline derivative, a 1-Alpha-amino-3-biphenyl-propanoic acid derivative, a 3-(Indol-3-yl)propenoic acid derivative, a spiro-heterocycle-imidazo-indeno-pyrazine-4-one derivative, a 2-heterocyclyl-3-indolylpropenoic acid derivative, a piperidinoalkyl heterocyclic ketone or alcohol compound, a pyrrolyl-tetrahydro-benzoquinoxaline-dione derivative, a 7-imidazolyl or dialkylamino,tetrahydroquinoxaline dione compound, a dibenzocycloheptene, a quinoxaline derivative, an aryl-thio-quinoxaline derivative, a heterocyclic substd. imidazolo-quinoxaline derivative, a 1,4-dihydro-quinoxaline-2,3-dione derivative, an oxa- or thia-aliphatically bridged quinoxaline derivative, an aza-aliphatically bridged quinoxaline-2,3-dione compound, a 3-amido- or 3-sulphamido-indole compound, a 3,5-disubstd. phenyl-naphthalene derivative, an imidazo (1,2-a)indeno (1,2-e) pyrazine-2-carboxylic acid derivative, a 3-phenyl-fused ring pyridine-dione derivative, a 2-phenyl-pyridazino-indole-dione derivative, a 4,6-disubstd. kynurenine compound. a phosphono derivative of imidazo(1,2-a)pyrimidine-2-carboxamide, a tetrahydro-quinoxaline-dione derivative with N-(alkyl)carbonyl-amino- or ureido group, a tryptophan derivative, a hetero-aliphatic or hetero-araliphatic substd. quinolone derivative, an imidazo-pyridine dicarboxylic acid derivative, a composition containing pyrazolo-quinoline derivatives, an ethanodihydrobenzoquinolizinium salt, an oxopyridinylquinoxaline derivative, an indeno-triazolopyrazin-4-one derivative, an imidazo-indeno-pyrazinone derivative. an imidazo-indeno-pyrazin-4-one derivative. an imidazo(1,2-a)pyrazine-4-one derivative, a 5H-indeno-pyrazine-2,3-dione

derivative, a phenyl-aminoalkyl-cyclopropane N,N-diethyl carboxamide compound, a dextranabinol derivative, a substituted chroman derivative, a sulphonamide quinazoline-2,4-dione compound, a 6- and 8-aza-, and 6,8-diaza-1,4-dihydro-quinoxaline-2,3-dione derivative, a substituted quinoline derivative, a tetrazolylalkyl cyclohexyl aminoalkanoic acid, a tricyclic indole 2-carboxylic acid derivative, a 6-substituted-7H-imidazo-8-pyrazinone derivative, a quinoxaline dione derivative or one of its radiolabelled compounds, a tricyclic pyridazinopyridine derivative, an N-substituted heterocyclidenemethyl-indole carboxylic acid derivative, a 3-aza-8-substituted-bicyclo(3.3.0)octane-2-carboxylic acid derivative, an ethano-heterocyclo-isoquinolinium salt, a phenyl alkanolamine derivative, a dihydrobenzothiadiazinedioxide carboxylic acid derivative, a methyl-butenylmethyl(hydroxy-propyl)carbazoledione, an imidazo pyrazinone derivative, an imidazo-(1,2-a)pyrazine-4-one, a benzazepine-dione derivative, disulfiram, a 3-(indol-3-yl)-propenoic acid derivative, a 1,2,3,4-tetrahydro-quinoline-2,3,4-trione-3 or 4-oxime compound, a peptide antagonist at NMDA receptors, a 2-amino-2-phenyl(alkyl)-acetic acid derivative, 6-halo-tryptophan or a 4-halo-kynurenine, a 6-tetrazolyl or isoxazolyl-decahydro-isoquinoline-3-carboxylic acid derivative, or an imidazolylbenzene or salts thereof.

19. The use according to any of claims 15 to 18, wherein the inhibitor is 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP), 2-(carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid (D-CPPene), 2-amino-5-pentanoic acid (AP5), 2-amino-7-heptanoic acid (AP7), selfotel (CGS19755), (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP101606), 5-nitro-6,7-dichloro-quinoxalinedione (ACEA1021), pyridazino[4,5-b]quinoline-1,4,10(5H)-trione, 7-chloro-2,3-dihydro-2-(4-methoxy-2-methylphenyl)-, monosodium salt (ZD9379), 1H-indole-2-carboxylic acid, 4,6-dichloro-3-[3-oxo-3-(phenylamino)-1-propenyl]-, monosodium salt (GV150526), 1-aminocyclopropanecarboxylic acid (ACPC), eliprodil (SL820715), lubeluzole, aminophosphovaleric acid, memantine (1-amino-3,5-dimethyladamantane), 3-(4-chlorophenyl)glutamic acid, (+)-beta-cyclazocine, (-)-beta-cyclazocine, DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 37849), 3-[(RS)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid, ketamine, phencyclidine, dextrorphan, dextromethorphan, N-(1-naphthyl)-N'-(3-ethylphenyl)-N'-methylguanidine hydrochloride (aptiganel, CNS1102), ifenprodil, (+)-alpha-phenyl-2-pyridine-ethanamide (FPL 15896AR), 5-aminocarbonyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (ADCI), bis(3-aminopropyl)nonanediamine (TE393), N-(3-aminopropyl)octanediamine, magnesium salts, 2R,4R,5S-(2-amino-4,5-(1,2-cyclohexyl)-7-

phosphono-heptanoic acid, 3-amino-1-hydroxy-2-pyrrolidinone (HA 966), D-(E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CGP 40116), (+/-)(E)-2-amino-4-methyl-5-phosphono-3-pentenoate ethylester (CGP39551), (+)-(3S,4S)-7-hydroxy-delta 6-tetrahydrocannabinol-(1,1)-dimethylheptyl (HU 211), (+)-1-methyl-1-phenyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (FR115427), (+/-)-6-phosphonomethyl-decahydroisoquinolin-3-carboxylic acid (LY274614), 3-isoquinolinecarboxylic acid, decahydro-6-(1H-tetrazol-5-ylmethyl)-, [3R-(3alpha,4alpha,6beta,8alpha)] (LY 233536), 2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoate (NPC 12626), (2R,4R,5S-2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid (NPC 17742), procyclidine, D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116), (+)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine, D,L-(E)-2-amino-4-propyl-5-phosphono-3-pentenoic acid, D-norvaline-4-oxo-5-phosphono (MDL-100453), cis-4-(phosphonomethyl)-2-piperidinecarboxylic acid, D,L-(E)-2-amino-4-propyl-5-phosphono-3-pentenoic acid (CGP 39653), conantokin-T, conantokin-G, γ -L-glutamyl-L-aspartate, (+/-)-(2SR,4SR)-4-(1H-tetrazol-5-ylmethyl)piperidine-2-carboxylic acid, DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 37849), (+/-)-3-carboxy-5-phosphono-1,2,3,4-tetrahydroisoquinoline (SC 48981), 1,2,3,4-tetrahydro-5-(2-phosphonoethyl)-3-isoquinolinecarboxylic acid, (1S,2S)-1-(4-Hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101,606,1), (3R,4S)-3-[4-(4-fluorophenyl)-4-hydroxypiperidin-1-yl]chroman-4,7-diol (12a, CP-283,097), ifenprodil derivatives 1-piperidineethanol,4-hydroxy-alpha-(4-hydroxyphenyl)-beta-methyl-4-phenyl-, [R-(R*,R*)] (CP-101,581) and 1-piperidineethanol,4-hydroxy-alpha-(4-hydroxyphenyl)-beta-methyl-4-phenyl-(alphaS,betaS) (CP-98,113), (+/-)-(E)-beta-cyclazocine, D- α -amino adipate (DAA), zinc salts, ibogaine, dextropropoxyphene, [3H]1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), 2-phenyl-1,3-propane-diol dicarbamate (felbamate), kynurenic acid, amantadine, Flupirtine (Katadolon), nitrous oxide (laughing gas), 4-{3-[4-(4-fluoro-phenyl)-3,6-dihydro-2H-pyridin-1-yl]-2-hydroxy-propoxy}-benzamide (Ro 8-4304), N1,N4,N8-tri-benzyl-spermidine (TB-3-4), 1(-)-3R,4aS,6R,8aR-6-(phosphonomethyl)-decahydroisoquinoline-3-carboxylic acid (LY235959), 2H-1,2,4-benzothiadiazine-1-dioxide-3-carboxylic acid (RPR 104632), dizocilpine maleate {(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine maleate} ((+)MK-801), 2R, 4R, 5S-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid) (NPC 17742), cis-(+/-)-4-[(2H-tetrazol-5-yl) methyl]piperidine-2-carboxylic acid (LY 233053), 2-amino-6-phosphonohexanoic acid, D-2-amino-5-phosphonovaleric acid (5-APV). (+)-2-Amino-N-ethyl-alpha-(3-methyl-2-thienyl)benzeneethanamine 2HCl (8319), desipramine, [3H]N-(1-(2-thienyl)-cyclohexyl)-3,4-piperidine (TCP), 4-(phosphonomethyl)-phenylglycine (PD 129635).

3-(phosphonomethyl)phenylalanine (PD 130527), tiletamine, arginine vasopressin, O-phosphohomoserine, D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CAS 137424-81-8), [±]-5-aminocarbonyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptan-5,10-imine (ADCI), 7-chlorokynurenate, ketoprofen, [(S)-Alpha-phenyl-2-pyridine-ethanamine dihydrochloride] ARL 15896AR, ((3S,4aR, 6R, 8aR)-6-[2-(1 H-tetrazol-5-yl)-ethyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3 -carboxylic acid) (LY293558).

20. The use according to any of claims 15 to 16, wherein the inhibitor is an NMDA receptor channel blocker.

21. The use according to claim 20, wherein the NMDA receptor channel blocker is dizocilpine (MK801), memantine, budipine, flupirtine, remacemide, phencyclidine, tiletamine, ketamine, carvedilol, aptiganel (CNS1102), remacemide (FPL12924AA), 7-hydroxy- Delta (6)-tetrahydrocannabinol 1,1-dimethylheptyl (Dexanabinol; HU211), 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), or their derivatives.

22. The use according to any of claims 1 to 2, 8 to 9, and 15 to 16 wherein the inhibitor is a glutamate release inhibitor.

23. The use according to claim 22, wherein the glutamate release inhibitor is i.e. riluzole, lamotrigine, diphenylhydantoin, tetrodotoxin, agatoxin-glutamate-release-inhibitor (AG-GI), [5-(2,3,5-trichlorophenyl)]-2,4-diamino-pyrimidine (BW1003C87), (R)-(-)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153) and 4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl)pyrimidine (BW619C89) or any other agent that decreases the release of glutamate from nerve endings and prevents glutamate from binding to its binding sites and from triggering the signal that would occur as a result of binding of glutamate to its binding sites.

24. The use according to any of claims 1 to 2, 8 to 9, and 15 to 16 wherein the inhibitor is a glutamate synthesis inhibitor.

25. The use according to claim 24, wherein the glutamate synthesis inhibitor is gabapentin, L-canaline, phenylsuccinate, spermidine, putrescine, gentamicin, orthovanadate, vanadyl sulphate, vanadyl acetylacetonate, methionine sulfoximine, chloroquine, amodiaquine, quinacrine,

chinidine, chinine, α -monofluoromethylputrescine and (R,R)-delta-methyl- α -acetylenic-putrescine, or any other agent which interacts with glutamate synthesis or metabolism and prevents activation of its receptors by glutamate.

26. The use according to any of claims 1 to 2, 8 to 9, and 15 to 16 wherein the inhibitor is an agent accelerating glutamate uptake.

27. The use according to claim 26, wherein the agent accelerating glutamate uptake is γ -glutamyl-transpeptidase, or any other agent which decreases synaptic concentration of glutamate by activating uptake mechanism for glutamate.

28. The use according to any of claims 1 to 2, 8 to 9, and 15 to 16 wherein the inhibitor is an agent that interacts with glutamate itself and prevents its binding to glutamate receptors.

29. The use according to claim 28, wherein the agent that interacts with glutamate is D-serine, D-cycloserine, γ -L-glutamylglutamate, N-phthalamoyl-L-glutaminic acid, (R,S)-2-amino-3-[5-tert-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid, α -N-acetylaspartylglutamate, 1-aminocyclopropanecarboxylic acid, aminocyclobutane carboxylic acid, (+,R)-3-amino-1-hydroxy-2-pyrrolidine (HA966) and D,L-threo-3-hydroxyaspartate, or any other agent which changes conformational state of glutamate and therefore decreases its binding to receptors. Furthermore such agents include soluble forms of AMPA, kainate or NMDA receptors or parts thereof which can be used to circulate and to bind to glutamate and therefore decrease its binding capability to the receptors.

30. The use according to any of claims 1 to 2, 8 to 9, and 15 to 16 wherein the inhibitor is a glutamate transporter activator that decreases the concentration of glutamate and prevents its binding to the AMPA, kainate or NMDA receptors.

31. The use according to claim 30, wherein the agent that blocks glutamate transporter is 12-O-tetradecanoylphorbol-13-acetate and phorbol-12-myristate 13-acetate, or any other agent which accelerates the function of glutamate transporters.

32. The use according to any of claims 1 to 2, 8 to 9, and 15 to 16 wherein the inhibitor is an antibody interacting with AMPA, kainate, or NMDA receptors of parts of it or with glutamate and prevents binding of glutamate to its receptors.
33. The use according to claim 32, wherein a preferred antibody which binds specifically to the AMPA, kainate or NMDA receptor or a part thereof, or to glutamate is monoclonal or polyclonal or derivative thereof.
34. The use according to any preceding claim wherein the inhibitor is combined with one or more of: a cytostatic agent (such as alkylating agents e.g. nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, busulfan, nitrosoureas, BCNU, CCNU, methyl-CCNU; such as antimetabolites e.g. antifolates, pyrimidine and purine analogs including e.g. methotrexate, 5-fluorouracil, azathioprine, cytosine arabinoside, 6-thioguanine, 6-mercaptopurine; such as natural products based anticancer drugs including e.g. doxorubicin, daunorubicin, daunomycin, actinomycin D, bleomycin, mitoxantrone, neocarzinostatin, procarbazine, mitomycin C, vinblastine, vincristine, etoposide; such as intercalating drugs e.g. cisplatin, carboplatin; and other anticancer drugs such as e.g. dacarbazine), an immunomodulating agent (e.g. corticosteroids as e.g. prednisone and methylprednisolone; interferons such as interferon- α (IFN- α), IFN- β , IFN- γ , and other potential modulators such as e.g. interleukins (IL-1 - IL7)); and with physical measures such as irradiation, or hyperthermia. The agents of present invention can also be combined with mono- or polyclonal antibodies, antisense therapeutics, cancer vaccines, and gene therapy.
35. A method of screening for an agent useful in treating cancer, determining whether or not said agent is an inhibitor of the interaction of glutamate with the AMPA, kainate, or NMDA receptor complex on tumor cells.
36. A pharmaceutical composition comprising an inhibitor as described in any of claims 1 to 34 and a pharmaceutically acceptable carrier.
37. A combined preparation of an inhibitor as described in any of claims 1 to 34 and one or more of: a cytostatic agent (such as alkylating agents e.g. nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, busulfan, nitrosoureas, BCNU, CCNU, methyl-CCNU; such as antimetabolites e.g. antifolates, pyrimidine and purine analogs including e.g. methotrexate, 5-

fluorouracil, azathioprine, cytosine arabinoside, 6-thioguanine, 6-mercaptopurine; such as natural products based anticancer drugs including e.g. doxorubicin, daunorubicin, daunomycin, actinomycin D, bleomycin, mitoxantrone, neocarzinostatin, procarbazine, mitomycin C, vinblastine, vincristine, etoposide; such as intercalating drugs e.g. cisplatin, carboplatin; and other anticancer drugs such as e.g. dacarbazine), an immunomodulating agent (e.g. corticosteroids as e.g. prednisone and methylprednisolone; interferons such as interferon- α (IFN- α), IFN- β , IFN- γ , and other potential modulators such as e.g. interleukins (IL-1 - IL7).

ABSTRACT

New Use of Glutamate Antagonists for the Treatment of Cancer

Pharmaceutical Compositions And Their Uses

New therapies can be devised based upon a demonstration of the role of glutamate in the pathogenesis of cancer. Inhibitors of the interaction of glutamate with the AMPA, kainate, or NMDA receptor complexes are likely to be useful in treating cancer and can be formulated as pharmaceutical compositions. They can be identified by appropriate screens.

fluorouracil, azathioprine, cytosine arabinoside, 6-thioguanine, 6-mercaptopurine; such as natural products based anticancer drugs including e.g. doxorubicin, daunorubicin, daunomycin, actinomycin D, bleomycin, mitoxantrone, neocarzinostatin, procarbazine, mitomycin C, vinblastine, vincristine, etoposide; such as intercalating drugs e.g. cisplatin, carboplatin; and other anticancer drugs such as e.g. dacarbazine), an immunomodulating agent (e.g. corticosteroids as e.g. prednisone and methylprednisolone; interferons such as interferon- α (IFN- α), IFN- β , IFN- γ , and other potential modulators such as e.g. interleukins (IL-1 - IL7).

ABSTRACT

New Use of Glutamate Antagonists for the Treatment of Cancer

Pharmaceutical Compositions And Their Uses

New therapies can be devised based upon a demonstration of the role of glutamate in the pathogenesis of cancer. Inhibitors of the interaction of glutamate with the AMPA, kainate, or NMDA receptor complexes are likely to be useful in treating cancer and can be formulated as pharmaceutical compositions. They can be identified by appropriate screens.



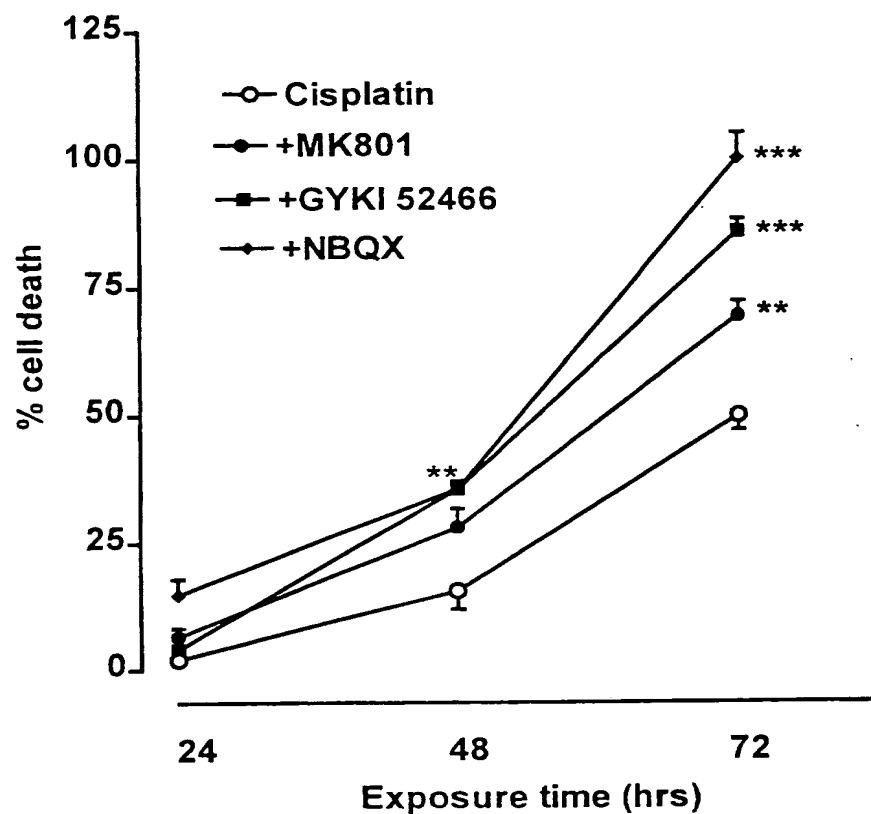


FIGURE 1 shows that the the NMDA antagonist MK801 (10 μ M), and the AMPA receptor antagonists GYKI 52466 (10 μ M) or NBQX (10 μ M) significantly increase toxicity of cisplatin (10 μ M) against the human neuroblastoma cell line SK-N-AS. Data represent the mean \pm SEM of necrotic cells visualized by trypan blue staining (n = 5/group). The potentiating effect of all three glutamate receptor antagonists was most prominent after an exposure time of 72 h. *P<0.05; **P<0.01; ***P<0.001, Student's t test.

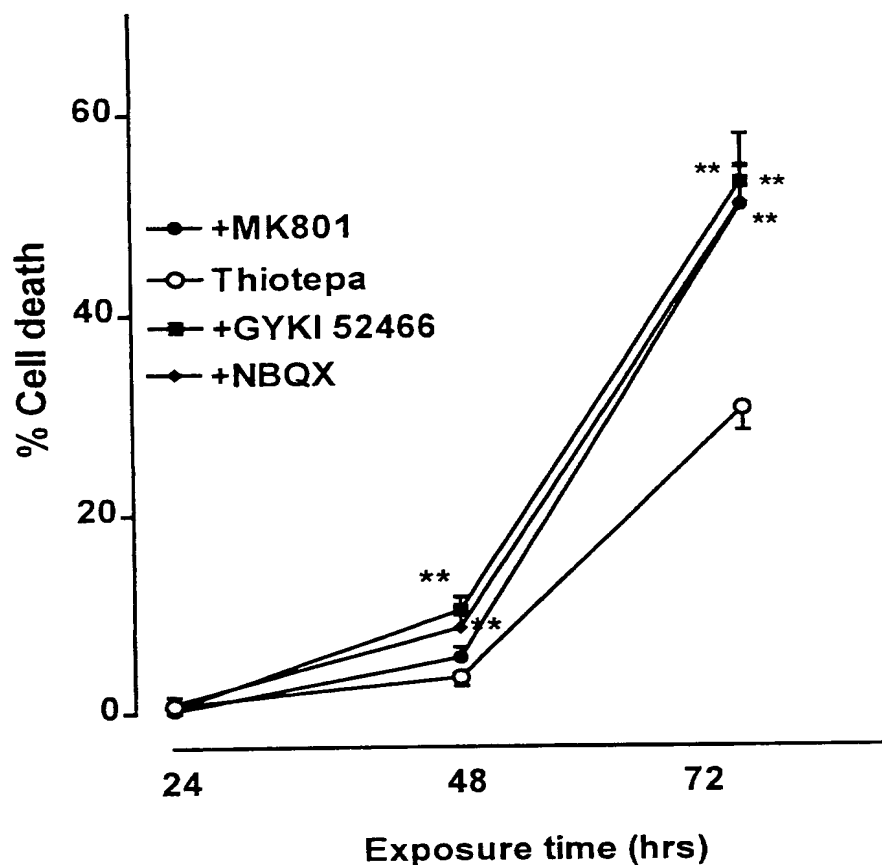


FIGURE 2 shows that MK801 (10 μ M), GYKI 52466 (10 μ M) or NBQX (10 μ M) potentiate toxicity of thiotepa (10 μ M) against the tumor cell line SK-N-AS. Data represent the mean \pm SEM of necrotic cells visualized by trypan blue staining (n = 5/group). The potentiating effect of the NMDA- and both AMPA antagonists is most prominent after an exposure time of 72 h. *P<0.05; **P<0.01; ***P<0.001, Student's t test.

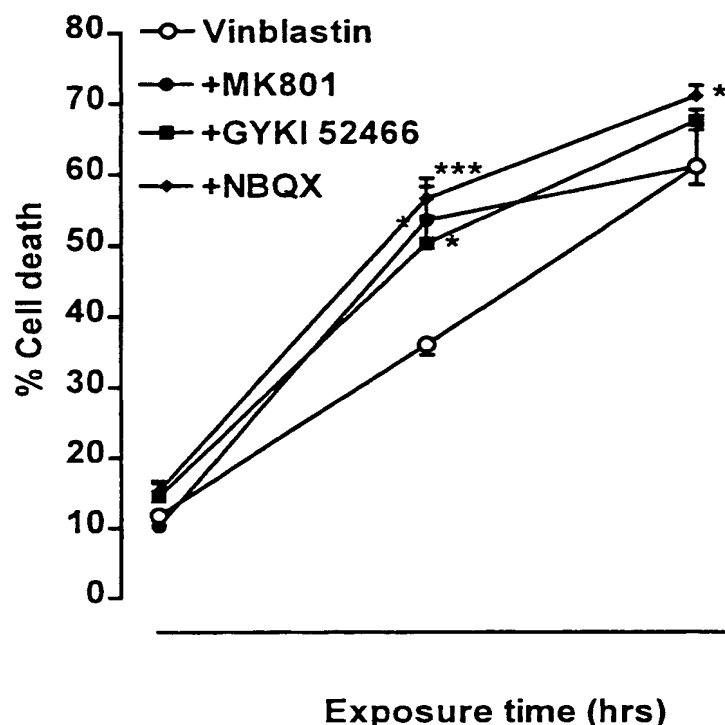


FIGURE 3 shows that MK 801 (10 μ M), GYKI 52466 (10 μ M) or NBQX (10 μ M) potentiate toxicity of vinblastin (1 μ M) against the tumor cell line SK-N-AS. Data represent the mean \pm SEM of necrotic cells visualized by trypan blue staining (n = 5/group). The potentiating effect of the NMDA- and both AMPA antagonists is most prominent after an exposure time of 48 hrs. *P<0.05;**P<0.01;***P<0.001, Student's t test.

Examples

The tumor cell line SK-N-AS has been obtained from human neuroblastoma, a primary neuroectodermal tumor. Agents active against tumor growth *in vitro* have proved to also be effective anticancer agents *in vivo*.

The surprising observation that the tumoricidal effect of three different cytostatic agents, cisplatin, vinblastin and thiotepa is significantly enhanced by two different AMPA antagonists, GYKI 52466 and NBQX (2,3-dihydroxy-6-nitro-7-sulfaoylbenzo-(F)-quinoxaline) and the NMDA antagonist MK 801 is described in the following paragraphs.

